

## WHAT IS CLAIMED IS:

1. A cell culture comprising human cells wherein at least about 10% of said human cells are definitive endoderm cells, said definitive endoderm cells being multipotent cells that can differentiate into cells of the gut tube or organs derived therefrom.
2. The cell culture of claim 1, wherein at least about 50% of said human cells are definitive endoderm cells.
3. The cell culture of claim 1, wherein at least about 80% of said human cells are definitive endoderm cells.
4. The cell culture of claim 1, wherein said definitive endoderm cells express a marker selected from the group consisting of SOX17 and CXCR4.
5. The cell culture of claim 4, wherein the expression of a marker selected from the group consisting of SOX17 and CXCR4 is greater than the expression of a marker selected from the group consisting of OCT4, alpha-fetoprotein (AFP), Thrombomodulin (TM), SPARC and SOX7 in said definitive endoderm cells.
6. The cell culture of claim 4, wherein said definitive endoderm cells do not express a marker selected from the group consisting of OCT4, AFP, TM, SPARC and SOX7.
7. The cell culture of claim 4, wherein said definitive endoderm cells express a marker selected from the group consisting of MIXL1, GATA4 and HNF3b.
8. The cell culture of claim 4, wherein said definitive endoderm cells express a marker selected from the group consisting of FGF17, VWF, CALCR, FOXQ1, CMKOR1 and CRIP1.
9. The cell culture of claim 1, wherein said definitive endoderm cells express SOX17 and CXCR4.
10. The cell culture of claim 9, wherein the expression of SOX17 and CXCR4 is greater than the expression of OCT4, AFP, TM, SPARC and SOX7 in said definitive endoderm cells.
11. The cell culture of claim 9, wherein said definitive endoderm cells do not express OCT4, AFP, TM, SPARC and SOX7.
12. The cell culture of claim 9, wherein said definitive endoderm cells express MIXL1, GATA4 and HNF3b.
13. The cell culture of claim 9, wherein said definitive endoderm cells express a marker selected from the group consisting of FGF17, VWF, CALCR, FOXQ1, CMKOR1 and CRIP1.
14. The cell culture of claim 1, wherein at least about 2 definitive endoderm cells are present for about every 1 pluripotent cell in said cell culture.

15. The cell culture of claim 14, wherein said pluripotent cell comprises an embryonic stem cell.
16. The cell culture of claim 15, wherein said embryonic stem cell is derived from a tissue selected from the group consisting of the morula, the inner cell mass (ICM) of an embryo and the gonadal ridges of an embryo.
17. The cell culture of claim 1 further comprising a medium which comprises less than about 10% serum.
18. The cell culture of claim 1 further comprising a growth factor of the Nodal/Activin subgroup of the TGF $\beta$  superfamily.
19. The cell culture of claim 1, further comprising a growth factor selected from the group consisting of Nodal, Activin A, Activin B and combinations thereof.
20. A cell population comprising cells wherein at least about 90% of said cells are human definitive endoderm cells, said human definitive endoderm cells being multipotent cells that can differentiate into cells of the gut tube or organs derived therefrom.
21. The cell population of claim 20, wherein at least about 95% of said cells are human definitive endoderm cells.
22. The cell population of claim 20, wherein at least about 98% of said cells are human definitive endoderm cells.
23. The cell population of claim 20, wherein said human definitive endoderm cells express a marker selected from the group consisting of SOX17 and CXCR4.
24. The cell population of claim 23, wherein the expression of a marker selected from the group consisting of SOX17 and CXCR4 is greater than the expression of a marker selected from the group consisting of OCT4, AFP, TM, SPARC and SOX7 in said human definitive endoderm cells.
25. The cell population of claim 23, wherein said human definitive endoderm cells do not express a marker selected from the group consisting of OCT4, AFP, TM, SPARC and SOX7.
26. The cell population of claim 23, wherein said human definitive endoderm cells express a marker selected from the group consisting of MIXL1, GATA4 and HNF3b.
27. The cell population of claim 23, wherein said definitive endoderm cells express a marker selected from the group consisting of FGF17, VWF, CALCR, FOXQ1, CMKOR1 and CRIP1.
28. The cell population of claim 20, wherein said human definitive endoderm cells express SOX17 and CXCR4.
29. The cell population of claim 28, wherein the expression of SOX17 and CXCR4 is greater than the expression of OCT4, AFP, TM, SPARC and SOX7 in said human definitive endoderm cells.

30. The cell population of claim 28, wherein said human definitive endoderm cells do not express OCT4, AFP, TM, SPARC and SOX7.
31. The cell population of claim 28, wherein said human definitive endoderm cells express MIXL1, GATA4 and HNF3b.
32. The cell population of claim 28, wherein said definitive endoderm cells express a marker selected from the group consisting of FGF17, VWF, CALCR, FOXQ1, CMKOR1 and CRIP1.
33. The cell population of claim 20, wherein at least about 2 human definitive endoderm cells are present for about every 1 pluripotent cell in said cell population.
34. The cell population of claim 33, wherein said pluripotent cell comprises an embryonic stem cell.
35. The cell population of claim 34, wherein said embryonic stem cell is derived from a tissue selected from the morula, the ICM of an embryo and the gonadal ridges of an embryo.
36. A method of producing definitive endoderm cells, said method comprising the steps of:
- obtaining a cell population comprising pluripotent human cells;
  - providing said cell population with at least one growth factor of the TGF $\beta$  superfamily in an amount sufficient to promote differentiation of said pluripotent cells to definitive endoderm cells, said definitive endoderm cells being multipotent cells that can differentiate into cells of the gut tube or organs derived therefrom; and
  - allowing sufficient time for definitive endoderm cells to form, wherein said sufficient time for definitive endoderm cells to form has been determined by detecting the presence of definitive endoderm cells in said cell population.
37. The method of claim 36, wherein at least about 10% of said pluripotent cells differentiate into definitive endoderm cells.
38. The method of claim 36, wherein at least about 50% of said pluripotent cells differentiate into definitive endoderm cells.
39. The method of claim 36, wherein at least about 70% of said pluripotent cells differentiate into definitive endoderm cells.
40. The method of claim 36, wherein at least about 80% of said pluripotent cells differentiate into definitive endoderm cells.
41. The method of claim 36, wherein detecting the presence of definitive endoderm cells in said cell population comprises detecting the expression of at least one marker selected from the group consisting of SOX17 and CXCR4 and at least one marker from the group consisting of OCT4, AFP, TM, SPARC and SOX7 in cells of said cell population, wherein the expression of a marker selected from the group consisting of SOX17 and CXCR4 is greater than the expression of

a marker selected from the group consisting of OCT4, AFP, TM, SPARC and SOX7 in said definitive endoderm cells.

42. The method of claim 36, wherein detecting the presence of definitive endoderm cells in said cell population comprises detecting the expression of at least one marker selected from the group consisting of SOX17 and CXCR4 and at least one marker from the group consisting of AFP, TM, and SOX7 in cells of said cell population, wherein the expression of a marker selected from the group consisting of SOX17 and CXCR4 is greater than the expression of a marker selected from the group consisting of AFP, TM, and SOX7 in said definitive endoderm cells.

43. The method of claim 42, wherein the expression of at least one of said markers is determined by Q-PCR.

44. The method of claim 42, wherein the expression of at least one of said markers is determined by immunocytochemistry.

45. The method of claim 36, wherein detecting the presence of definitive endoderm cells in said cell population comprises detecting the expression of at least one marker selected from the group consisting of FGF17, VWF, CALCR, FOXQ1, CMKOR1 and CRIP1 and at least one marker from the group consisting of OCT4, AFP, TM, SPARC and SOX7 in cells of said cell population, wherein the expression of a marker selected from the group consisting of FGF17, VWF, CALCR, FOXQ1, CMKOR1 and CRIP1 is greater than the expression of a marker selected from the group consisting of OCT4, AFP, TM, SPARC and SOX7 in said definitive endoderm cells.

46. The method of claim 36, wherein said at least one growth factor is of the Nodal/Activin subgroup of the TGF $\beta$  superfamily.

47. The method of claim 46, wherein said at least one growth factor is selected from the group consisting of Nodal Activin A, Activin B and combinations thereof.

48. The method of claim 47, wherein said at least one growth factor is Nodal.

49. The method of claim 47, wherein said at least one growth factor is Activin A.

50. The method of claim 47, wherein said at least one growth factor is Activin B.

51. The method of claim 36, wherein a plurality of growth factors of the TGF $\beta$  superfamily is provided.

52. The method of claim 51, wherein said plurality of growth factors comprises Nodal Activin A and Activin B.

53. The method of claim 36, wherein said at least one growth factor is provided in a concentration of at least about 10 ng/ml.

54. The method of claim 36, wherein said at least one growth factor is provided in a concentration of at least about 100 ng/ml.

55. The method of claim 36, wherein said at least one growth factor is provided in a concentration of at least about 500 ng/ml.
56. The method of claim 36, wherein said at least one growth factor is provided in a concentration of at least about 1000 ng/ml.
57. The method of claim 36, wherein said at least one growth factor is provided in a concentration of at least about 5000 ng/ml.
58. The method of claim 36, wherein said cell population is grown in a medium comprising less than about 10% serum.
59. The method of claim 36, wherein said pluripotent cells comprise stem cells.
60. The method of claim 59, wherein said pluripotent cells comprise embryonic stem cells.
61. The method of claim 60, wherein said embryonic stem cells are derived from a tissue selected from the group consisting of the morula, the ICM of an embryo and the gonadal ridges of an embryo.
62. A definitive endoderm cell produced by the method of claim 36.
63. A method of producing a cell population enriched in definitive endoderm cells, said method comprising the steps of:
- differentiating cells in a population of pluripotent human cells so as to produce definitive endoderm cells, said definitive endoderm cells being multipotent cells that can differentiate into cells of the gut tube or organs derived therefrom;
  - providing to said cell population a reagent which binds to a marker expressed in said definitive endoderm cells but which is not substantially expressed in other cell types present in said cell population; and
  - separating said definitive endoderm cells bound to said reagent from said other cell types present in said cell population, thereby producing a cell population enriched in definitive endoderm cells.
64. The method of claim 63, wherein the differentiating step further comprises obtaining a cell population comprising pluripotent human cells, providing said cell population with at least one growth factor of the TGF $\beta$  superfamily in an amount sufficient to promote differentiation of said pluripotent cells to definitive endoderm cells, said definitive endoderm cells being multipotent cells that can differentiate into cells of the gut tube or organs derived therefrom, and allowing sufficient time for definitive endoderm cells to form, wherein said sufficient time for definitive endoderm cells to form has been determined by detecting the presence of definitive endoderm cells in said cell population.
65. The method of claim 63, wherein detecting comprises detecting the expression of at least one marker selected from the group consisting of SOX17 and CXCR4 and at least one

marker from the group consisting of OCT4, AFP, TM, SPARC and SOX7 in cells of said cell population, wherein the expression of a marker selected from the group consisting of SOX17 and CXCR4 is greater than the expression of a marker selected from the group consisting of OCT4, AFP, TM, SPARC and SOX7 in said definitive endoderm cells.

66. The method of claim 63, wherein detecting comprises detecting the expression of at least one marker selected from the group consisting of SOX17 and CXCR4 and at least one marker from the group consisting of AFP, TM, and SOX7 in cells of said cell population, wherein the expression of a marker selected from the group consisting of SOX17 and CXCR4 is greater than the expression of a marker selected from the group consisting of AFP, TM, and SOX7 in said definitive endoderm cells.

67. The method of claim 63, wherein detecting comprises detecting the expression of at least one marker selected from the group consisting of FGF17, VWF, CALCR, FOXQ1, CMKOR1 and CRIP1 and at least one marker from the group consisting of OCT4, AFP, TM, SPARC and SOX7 in cells of said cell population, wherein the expression of a marker selected from the group consisting of FGF17, VWF, CALCR, FOXQ1, CMKOR1 and CRIP1 is greater than the expression of a marker selected from the group consisting of OCT4, AFP, TM, SPARC and SOX7 in said definitive endoderm cells.

68. The method of claim 63, wherein at least about 95% of said cells are definitive endoderm cells.

69. The method of claim 63, wherein at least about 98% of said cells are definitive endoderm cells.

70. The method of claim 63, wherein said marker is CXCR4.

71. The method of claim 63, wherein said reagent is an antibody

72. The method of claim 71, wherein said antibody has affinity for CXCR4.

73. An enriched population of definitive endoderm cells produced by the method of claim 63.

74. The cell culture of any one of claims 4 or 9, wherein said definitive endoderm cells do not significantly express a marker selected from the group consisting of OCT4, AFP, TM, SPARC and SOX7.

75. The cell population of any one of claims 23 or 28, wherein said definitive endoderm cells do not significantly express a marker selected from the group consisting of OCT4, AFP, TM, SPARC and SOX7.